EXHIBIT A136

Relation of Particle Dimension to Carcinogenicity in Amphibole Asbestoses and Other Fibrous Minerals [1,2]

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ABSTRACT—In 72 experiments, durable minerals in the form of particles on respirable size and of wide chemical and structural varieties, were implanted in the pleurae of outbred female Osborne-Mendel rats for periods of more than 1 year. The incidence of induced malignant mesenchymal neoplasms correlated well with the dimensional distribution of the particles. The probability of pleural sarcoma correlated best with the number of fibers that measured 0.25 μm or less in diameter and more than 8 μm in length, but relatively high correlations were also noted with fibers in other size categories having diameters up to 1.5 μm and lengths greater than 4 μm. Morphologic observations indicated that short fibers and large-diameter fibers were inactivated by phagocytosis and that negligible phagocytosis of long, thin fibers occurred. The wide variety of compounds used in these experiments suggested that the carcinogenicity of fibers depended on dimension and durability rather than on physicochemical properties.—JNCI 1981; 67:965–975.

Work in several laboratories has indicated that diverse varieties of minerals are carcinogenic when applied directly to the pleura of the rat or hamster in the form of microscopic fibers, i.e., particles with dimensional aspect ratios of 3:1 or greater (*1–9*). The same minerals are much less carcinogenic when applied at equal weight and size in nonfibrous form. Further, preliminary experiments indicate that carcinogenicity correlates best with increasing numbers of fibers having both diameters of 0.25 μm or less and lengths of more than 8 μm and that the correlation diminishes with fibers of greater diameter or lesser length. Consequently, a reasonable conclusion is that the long, thin, fibrous structure is critical to the carcinogenicity of these minerals. Studies on fibrous samples within very narrow dimensional ranges would be valuable in the establishment of this hypothesis, but these ideal samples are not available. Consequently, we are faced with the correlation of carcinogenicity with fiber samples of widely mixed dimension. The purpose of this report is to correlate our best estimate of fibrous dimension with carcinogenicity for all those minerals that we have studied that are both durable and within the size range of respirable particles. This involves 72 experiments with minerals of wide chemical and structural variety. Of special interest are the data on the amphibole asbestoses: amosite, tremolite, and crocidolite, though estimates of the dimensions of the asbestoses are especially liable to error. Chrysotile, although as carcinogenic as the amphiboles at comparable dimensions, could not be included since it has proved difficult to be measured with any degree of precision.

MATERIALS AND METHODS

None of the methods were appreciably different from those described in earlier papers (*4, 6, 9–11*). Consequently, only modifications of methods are detailed here. A standard 40-mg dose of particles uniformly dispersed in hardened gelatin was applied by open thoracotomy directly to the left pleural surface of 12- to 20-week-old, outbred female Osborne-Mendel rats. In each experiment, 30–50 rats were treated and followed for 2 years, at which time the survivors were killed. All rats were necropsied and all lesions examined histologically. A positive response was the occurrence of pleural sarcomas that resembled the mesenchymal mesotheliomas of man, developing after the 1st year (*12*). Three types of controls were considered: untreated rats, rats that received thoracotomies but no pleural implant, and rats with pleural implants of nonfibrous material. There were two types of spontaneous tumors that could cause confusion: the fibrosarcomas of left mammary glands and the subcutaneous fibrosarcomas induced by suture material. Vigilance and early surgical removal accounted for most mammary tumors; the use

ABBREVIATIONS USED: alumin=aluminum oxide; attapul=attapulgite(s); crocid=crocidolite(s); dawson=dawsonite(s); halloy=halloysite(s); UICC=International Union Against Cancer; wollaston=wollastonite(s).

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[2] The guidelines for the care and use of laboratory animals were followed as set forth by the Committee on Revision of the Guide for Laboratory Animal Facilities; by the Guide for the Care and Use of Laboratory Animal Resources, the National Research Council; and by the National Institutes of Health.

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of synthetic, biodegradable, polyglycolic acid sutures largely eliminated suture sarcomas. An equivocal diagnosis for the origin of a tumor was necessary in less than 1% of the tumors. The probability of pleural sarcoma in each experiment was calculated by an actuarial life table method that accounts for early deaths without pleural sarcoma and provides a good means of making quantitative comparisons of one experiment with another. Details of this method are given in (*13, 14*).

The fibrous materials used in these experiments were mostly commercial products that were submitted by the manufacturers from an interest in their potential carcinogenicity. Consequently, they were used as received and were not especially refined except in our efforts to separate particles by size. None of the preparations appeared overtly contaminated by other materials when examined in the electron microscope. A few of the small-fibered subfractions of the fibrous materials were obtained by ball milling in a steel ball mill and consequently were contaminated with fragments of steel. In general, subfractions were obtained by simple gravimetric methods in aqueous media to separate fibers of different dimensions. These maneuvers included sedimentation, centrifugation, and filtration, which in some instances were also responsible for the reduction of the size of the particles but did not otherwise alter the particles physically or chemically. Eleven chemically and structurally different groups of fibers were available for study, and samples studied are listed in text-figure 1 and table 1. Six major groups of particles had multiple dimensional ranges; these include: crocidolites: (samples crocid 1-13), glasses (glass 1-22), aluminum oxide whiskers (alumin 1-8), talcs (talc 1-7), dawsonites (dawson 1-7), and wollastonites (wollaston 1-4). Seven additional types of particles had only one or two dimensional ranges. These were the amphibole asbestoses tremolite (tremolite 1, 2) and amosite, the clays attapulgite (attapul 1, 2) and halloysite (halloy 1, 2), crystals of silicon carbide and potassium titanate (titanate 1, 2), and nickel titanate (titanate 3). All of these materials have been described elsewhere (*4, 6, 10, 11, 15-18*), but the following information is pertinent.

Crocidolite (crocid 1-13).—These 13 samples of South African crocidolite (an amphibole asbestos) were from four different sources. Samples crocid 1, 3, and 9 were prepared in our laboratory from a single sample of hand-cobbed, unmilled ore. The ore sample was hand milled without exposure to any metallic materials and reduced to the approximate size of commercial crocidolite. Samples crocid 6, 7, 8, 11, 12, and 13 were all prepared in our laboratory by various milling, sedimentation, and flotation methods from a single lot of standard UICC crocidolite designated crocid 5. Differences in dimension were the result of different milling times. Crocid 5, the original UICC sample, has been characterized in (*19, 20-23*). Samples crocid 4 and 10 were specimens prepared in a commercial laboratory from a single separate sample of South African crocidolite and separated by centrifugation to obtain mutually exclusive size ranges from the same sample (*24*). The remaining sample, crocid 2, was obtained from Dr. J. C. Wagner (Medical Research Council Pneumoconiosis Unit, Penarth, Wales) as representative of the material used by him in his original experiments (*25*). It was our impression that any mechanical manipulation of these samples could both reduce the size of the particles by fragmentation and effectively increase the size of the particles by clumping. For this reason, probably the dimensional measurements on crocidolite are the least representative of all the fibers measured.

Glass (glass 1-22).—The first 18 of the 22 glasses were borosilicate glasses that have been previously reported and can be recognized from those publications by their letter designations (*4, 10*). Glasses 12, 14, 15, and 18 were preparations of typical large-diametered insulation glass fibers that were coated with a phenol-formaldehyde binder. In the early experiments, glass 18 was used as a control and also served as a vehicle for the implants. Glasses 19 and 20 were preparations of large-diametered fibrous glass that was leached to remove all elements except SiO_2. These two glasses were exceptionally fragile and contained many irregular fragments. Glasses 21 and 22 were large-diametered extruded fibers with a microcrystalline aluminum oxide content greater than 80% (glass 21) and with a microcrystalline zirconium oxide content greater than 90% (glass 22).

Aluminum oxide (alumin 1-8).—The 8 samples of aluminum oxide were all crystalline sapphire whiskers prepared by General Technologies Corporation, Reston, Va., or by Thermokinetics Fiber Incorporated, Nutley, N.J. (*15-18, 26*). All of the samples were processed and selected for dimensional ranges. Of the samples, 3 were exceptionally noteworthy. Sample alumin 8 was nonfibrous, sample alumin 3 was exceptionally fine but tended to cluster in nonfibrous balls, and sample alumin 4 contained whiskers of aluminum nitride as well as aluminum oxide.

Talcs (talc 1-7).—All seven talcs were refined raw materials for commercial products. Each was from a separate and diverse source and selected to include all extreme ranges of dimension. Platelike structure was consistent and was considered in the calculation of the volume (*15-18*).

Dawsonite (dawson 1-7).—The 7 dawsonite samples (crystalline dehydroxy sodium aluminum carbonate [$NaAl(OH)_2CO_3$] were from several sources. The characteristics and synthesis of dawsonite can be found in (*27, 28*). Samples dawson 2 and 3 were synthetic crystals prepared by a commercial company (for dawson 2) and by the Bureau of Mines, U.S. Department of Interior (for dawson 3). Sample dawson 4 was a natural crystalline dawsonite from the Olduvai Gorge, Tanzania. The remaining 4 samples (dawson 1, 5, 6, and 7) were synthetic crystals from a second commercial company. These 4 samples were especially crystallized and sorted to achieve narrow ranges of size.

(1) Titanate 1 — 95%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5			3.75	4.11	
>.25-.50	3.57	4.41	4.79	4.87	
>.10-.25	3.87	5.08	5.34	5.17	
>.05-.10	3.27	5.02	5.02	4.44	
>.01-.05					

(2) Titanate 2 — 100%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5			3.70	3.40	
>.25-.50	3.70	4.18	4.52	4.18	
>.10-.25	3.88	4.99	5.07	4.48	
>.05-.10	4.25	4.91	4.78	4.31	
>.01-.05	3.40				

(3) Si Carbide — 100%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0			1.82	1.35	
>2.5-4.0			1.82	1.65	1.35
>1.5-2.5		2.25	2.04	1.95	
>.50-1.5	2.92	3.08	3.03	3.24	1.35
>.25-.50	3.29	3.89	4.20	4.40	
>.10-.25	0.81	4.01	4.19	4.56	
>.05-.10		4.21	4.41	4.74	
>.01-.05	3.78	4.48	4.54	4.70	

(4) Dawson 5 — 100%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5			1.87	3.02	
>.50-1.5		2.18	1.87	3.50	
>.25-.50	3.24	4.23	4.25	4.53	
>.10-.25		2.83	3.73	4.93	
>.05-.10					
>.01-.05					

(5) Tremolite 1 — 100%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5		3.14	3.14	3.14	
>.50-1.5	3.84	4.47	3.92	3.92	
>.25-.50	4.10	4.35	3.14	3.14	
>.10-.25	4.20	4.14	3.44	3.44	
>.05-.10	3.69	3.54	3.14	3.14	
>.01-.05					

(6) Tremolite 2 — 100%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0			2.54	3.01	
>1.5-2.5		3.14	2.84	2.54	
>.50-1.5		4.05	3.68	3.77	
>.25-.50	3.44	3.86	3.38	3.31	
>.10-.25	4.09	4.04	3.38	2.84	
>.05-.10	3.14	2.84			
>.01-.05					

(7) Dawson 1 — 95%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0				2.14	
>1.5-2.5		1.44	1.44	3.00	1.75
>.50-1.5	1.92	1.92	2.22	3.68	
>.25-.50	1.75	3.16	3.32	4.25	
>.10-.25	2.62	3.70	3.90	4.66	
>.05-.10	3.40	3.62	3.62	2.62	
>.01-.05	3.57	3.57			

(8) Crocid. 1 — 94%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5			4.07		
>.25-.50		4.07		4.55	
>.10-.25	5.75	5.69	4.67	5.15	
>.05-.10	6.52	5.62	4.67	4.37	
>.01-.05	5.87	4.67			

(9) Crocid. 2 — 93%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5	3.53	4.53	4.37	4.30	
>.25-.50	4.22	4.83	4.70	4.22	
>.10-.25	5.55	5.57	4.73	4.00	
>.05-.10	5.71	4.96		4.00	
>.01-.05	4.57	3.83			

(10) Crocid. 3 — 93%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5			4.10	4.10	
>.25-.50			4.10	4.40	
>.10-.25	6.12	5.71	4.95	5.01	
>.05-.10	6.52	5.84	4.10		
>.10-.05	5.62	4.58	4.10		

(11) Amosite — 93%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0				2.13	
>1.5-2.5				2.43	2.13
>.50-1.5	2.13	3.17	3.36	3.54	2.61
>.25-.50	3.13	3.64	3.56	3.72	
>.10-.25	3.53	3.76	3.31	3.47	
>.05-.10	2.98	2.98	3.03	2.61	
>.01-.05					

(12) Crocid. 4 — 86%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5		2.76	3.24	3.84	3.06
>.25-.50		4.24	3.61	4.36	
>.10-.25	3.24	4.33	4.50	4.92	
>.05-.10	2.76	4.31	4.53	4.70	
>.01-.05	3.24	3.36	3.54	3.06	

(13) Glass 1 (MOL) — 85%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5		2.23	2.53	3.23	3.08
>.25-.50		3.08		4.95	
>.10-.25		2.93	3.35	4.53	
>.05-.10			3.93	4.79	
>.01-.05			3.46	4.65	

(14) Crocid. 5 — 78%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0			2.81		
>2.5-4.0			2.81		
>1.5-2.5		2.81	3.29	2.81	
>.50-1.5	3.59	4.19	3.42	3.77	2.81
>.25-.50	4.35	4.35	3.66	3.59	
>.10-.25	4.84	4.65	3.77	3.29	
>.05-.10	4.74	4.63	3.42		
>.01-.05	4.42	4.26	2.81		

(15) Glass 2 (M6D) — 77%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5		2.91	3.21	3.99	3.61
>.25-.50		3.38	2.91	4.11	
>.10-.25		3.69	3.61	4.02	
>.05-.10		4.08	3.69	3.81	
>.01-.05	3.51	3.51	3.21	3.38	

(16) Glass 3 (KL) — 74%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					0.67
>2.5-4.0			0.67	1.52	0.97
>1.5-2.5		1.45	0.67	2.03	2.19
>.50-1.5		2.95	2.40	3.42	2.74
>.25-.50	2.55	3.16		3.63	
>.10-.25	3.03	3.16	3.33	3.03	
>.05-.10	2.85	4.09	3.76	3.25	
>.01-.05	3.03	3.73	3.03	3.03	

(17) Glass 4 (M6L) — 71%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0				1.53	1.23
>1.5-2.5			1.84	2.76	2.13
>.50-1.5	2.81	2.05	2.59	3.70	2.90
>.25-.50	2.44	2.44	3.16	3.39	
>.10-.25	2.44	3.52	3.44	3.62	
>.05-.10	2.44	3.44	3.56	3.70	
>.01-.05		3.44	3.35	3.14	

(18) Alumin. 1 — 70%

Diameter (μm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.20	
>4.0-8.0			1.10	1.80	0.80
>2.5-4.0		1.20	1.82	1.90	1.40
>1.5-2.5			1.61	2.17	1.58
>.50-1.5	3.12	2.26	1.93	2.78	2.21
>.25-.50			0.80	2.42	
>.10-.25			2.57	3.51	
>.05-.10				2.73	
>.01-.05			1.73	2.68	

Diameter / Length

TEXT-FIGURE 1.—Fiber distribution by common log of the number of particles per microgram in each of 34 dimensional categories.

(19) Glass 5 (KW) 69%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				0.06	0.06
>4.0-8.0				0.36	0.76
>2.5-4.0				0.06	1.06
1.5-2.5		0.66	0.37	1.41	1.98
>.50-1.5	2.48	1.80	1.29	2.42	2.51
>.25-.50	1.06	0.67	1.70	2.75	
>.10-.25			1.67	2.71	
>.05-.10			1.67	2.51	
>.01-.05				2.21	

(20) Dawson. 7 68%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0				1.77	
>1.5-2.5		2.07	2.72	3.33	
>.50-1.5	1.77	3.01	3.26	3.81	
>.25-.50	3.11	4.18	4.10	4.25	
>.10-.25	2.72	4.40	4.64	4.70	
>.05-.10		3.70	3.42	2.72	
>.01-.05					

(21) Dawson. 4 66%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.87	
>4.0-8.0				2.08	
>2.5-4.0		1.27	1.66	1.81	
>1.5-2.5		2.22	1.57	2.24	
>.50-1.5	2.11	2.11	2.22	2.48	
>.25-.50	3.38	3.42	3.24	2.80	
>.10-.25		4.05	4.42	4.01	
>.05-.10	5.30	5.00	4.12		
>.01-.05	5.03	3.47	3.99		

(22) Dawson. 3 66%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0				1.99	
>2.5-4.0					
>1.5-2.5				2.29	
>.50-1.5		2.69	3.07	3.33	
>.25-.50	4.18	4.50	4.38	4.87	
>.10-.25	3.91	4.74	4.41	5.31	
>.05-.10	5.85	5.18	4.88	5.29	
>.01-.05	5.90	5.17	4.28	5.14	

(23) Glass 6 (M6W) 84%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0				1.10	1.58
>2.5-4.0		1.10	1.09	1.95	1.10
>1.5-2.5		1.10	2.18	2.74	2.01
>.50-1.5	2.28	3.17	3.21	3.69	2.84
>.25-.50	1.40	2.84	3.11	3.54	
>.10-.25	1.70	3.22	3.25	3.69	
>.05-.10	3.10	3.55	3.47	3.76	
>.01-.05	2.71	2.95	2.71	2.80	

(24) Crocid. 6 63%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5					
>.50-1.5	3.47	4.56	4.32	4.02	
>.25-.50	4.17	5.07	4.71	4.45	
>.10-.25	5.10	5.45	4.86	4.57	
>.05-.10	4.74	4.90	4.21	3.47	
>.01-.05	3.65	4.07			

(25) Crocid. 7 56%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0				0.91	1.21
>2.5-4.0					1.51
>1.5-2.5				1.86	2.25
>.50-1.5			0.91	2.02	2.29
>.25-.50		1.69	1.69	2.34	
>.10-.25		2.05	1.91	2.51	
>.05-.10		1.86	1.91	2.05	
>.01-.05			9.91	0.91	

(26) Crocid. 8 53%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0			3.45		
>1.5-2.5		3.75	3.45		
>.50-1.5	4.45	4.75	3.75		
>.25-.50	5.08	4.86	3.75		
>.10-.25	5.61	5.09			
>.05-.10	4.55	4.59			
>.01-.05	5.23	4.15			

(27) Alumin. 2 44%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.32	0.37
>4.0-8.0			0.91	1.32	0.37
>2.5-4.0		1.07	1.15	1.53	1.02
>1.5-2.5		1.48	1.18	1.61	1.18
>.50-1.5	2.37	2.04	1.60	2.37	1.83
>.25-.50	1.99	1.11	1.70	3.02	
>.10-.25		1.20	1.50	2.89	
>.05-.10				1.98	
>.01-.05		1.20	1.20	1.20	

(28) Alumin. 3 41%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.72	
>4.0-8.0			1.19	1.75	0.19
>2.5-4.0		1.09	1.57	1.82	
>1.5-2.5		1.59	1.70	1.70	
>.50-1.5	2.36	2.42	2.33	2.61	0.67
>.25-.50		0.97	1.15	1.49	
>.10-.25	0.97	2.32	2.19	2.41	
>.05-.10		1.27	0.97	1.59	
>.01-.05					

(29) Crocid. 9 33%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5				3.13	3.13
>.50-1.5	3.13			3.13	
>.25-.50	3.43	3.13			
>.10-.25	4.51	4.25	3.43		
>.05-.10	5.24	4.92	4.25	4.09	
>.01-.05	5.48	5.14	4.34	3.74	

(30) Wollaston. 1 31%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0				2.11	
>2.5-4.0		2.11	2.81	3.19	
>1.5-2.5		3.11	3.06	2.11	
>.50-1.5	3.15	3.76	3.15	2.59	
>.25-.50	3.15	3.25	2.11		
>.10-.25	3.28	3.22	2.41		
>.05-.10	3.41	3.28			
>.01-.05					

(31) Alumin. 4 28%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.75	
>4.0-8.0			1.88	1.98	
>2.5-4.0		2.15	2.48	2.15	
>1.5-2.5		2.80	2.30	2.37	1.28
>.50-1.5	2.92	2.83	2.45	2.75	0.98
>.25-.50	3.57	3.01	3.19	2.83	
>.10-.25		2.29	1.99	2.29	
>.05-.10			2.29		
>.01-.05		1.99	2.69	2.29	

(32) Crocid. 10 37%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0		3.10			
>1.5-2.5		3.10		3.57	
>.50-1.5	3.79	3.57	3.40	3.10	
>.25-.50	4.69	4.57	3.57		
>.10-.25	5.03	5.01	3.79	3.10	
>.05-.10	5.13	4.59	3.10		
>.01-.05	4.96	4.46			

(33) Alumin. 5 22%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0			1.78	2.08	
>2.5-4.0		1.48	2.18	2.08	
>1.5-2.5		2.66	2.71	2.59	
>.50-1.5	3.59	3.77	3.41	3.26	1.48
>.25-.50	3.55	4.24	4.22	3.92	
>.10-.25		4.13	4.24	3.57	
>.05-.10		4.02	3.92	3.23	
>.01-.05	3.00	3.70	2.52		

(34) Glass 20 (RT) 22%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					0.88
>4.0-8.0					0.98
>2.5-4.0					
>1.5-2.5					
>.50-1.5					
>.25-.50					
>.10-.25					
>.05-.10					
>.01-.05					

(35) Glass 7 (KCP) 21%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				0.97	
>4.0-8.0			1.81	2.01	1.81
>2.5-4.0		1.44	2.05	1.81	
>1.5-2.5		2.05	2.17	2.31	0.97
>.50-1.5	3.00	3.59	3.17	2.85	2.01
>.25-.50	3.24	3.88	3.10	2.55	
>.10-.25	3.24	3.28	3.10	2.50	
>.05-.10	2.50	2.90			
>.01-.05	2.20	2.98	2.67		

(36) Wollaston. 3 19%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0			2.58	2.28	
>2.5-4.0		2.58	2.58	2.58	
>1.5-2.5		3.58	3.12	2.76	
>.50-1.5	3.12	4.09	3.46		
>.25-.50	3.32	3.32	2.28		
>.10-.25	3.18	3.32			
>.05-.10	3.42	3.48			
>.01-.05					

Length

TEXT-FIGURE 1 (*continued*).—Fiber distribution by common log of the number of particles per microgram in each of 34 dimensional categories.

(37) Halloy. 1 20%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					
> 2.5-4.0					
> 1.5-2.5					
> .50-1.5					
> .25-.50		5.26			
> .10-.25		5.95	4.95		
> .05-.10	6.47	6.98	6.16		
> .01-.05	6.07	6.21			

(38) Halloy. 2 23%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					
> 2.5-4.0					
> 1.5-2.5			4.51		
> .50-1.5		4.51			
> .25-.50		4.51			
> .10-.25	5.36	5.47	4.51		
> .05-.10	6.16	6.14	4.81		
> .01-.05	6.33	5.89			

(39) Glass 8 (KUP) 19%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0			1.26	2.04	1.74
> 2.5-4.0		2.11	2.52	2.31	1.26
> 1.5-2.5		2.73	2.71	2.44	
> .50-1.5	2.88	3.93	3.54	3.19	1.96
> .25-.50	3.36	3.54	2.84	2.57	
> .10-.25	3.36	3.50	2.61	2.31	
> .05-.10	3.17	3.32	2.63	2.80	
> .01-.05		2.31		2.31	

(40) Crocid. 11 19%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0				2.15	
> 4.0-8.0			1.72	2.50	
> 2.5-4.0		2.38	2.42		
> 1.5-2.5		3.03	1.72	1.42	
> .50-1.5	3.15	2.98	1.90		
> .25-.50	3.05	1.90			
> .10-.25	2.97				
> .05-.10	2.02				
> .01-.05					

(41) Glass 19 (RD) 15%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0			2.26	2.73	
> 2.5-4.0		1.56	1.73		
> 1.5-2.5		2.16	1.26		
> .50-1.5	2.56	2.92			
> .25-.50	2.67	2.37			
> .10-.25					
> .05-.10	2.43	1.26			
> .01-.05					

(42) Glass 9 (M8L) 14%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					0.17
> 2.5-4.0			0.77	1.73	2.02
> 1.5-2.5		1.49	1.12	2.11	2.27
> .50-1.5		2.60	1.45	2.35	2.42
> .25-.50		1.85		2.38	
> .10-.25				1.85	
> .05-.10					
> .01-.05					

(43) Alumin. 6 13%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0				0.37	0.22
> 4.0-8.0			0.12	1.22	1.44
> 2.5-4.0		0.48		0.67	0.70
> 1.5-2.5		0.82	0.67	1.54	1.49
> .50-1.5	0.75	1.22	0.12	0.97	0.63
> .25-.50	2.10	0.82	0.22	1.84	
> .10-.25				0.82	
> .05-.10					
> .01-.05					

(44) Dawson. 6 13%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					
> 2.5-4.0					
> 1.5-2.5					
> .50-1.5					
> .25-.50					
> .10-.25	6.17	6.47			
> .05-.10	6.96	6.86			
> .01-.05	7.86	6.78			

(45) Dawson. 2 12%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0			2.44	2.69	
> 2.5-4.0		2.94	2.82	2.44	
> 1.5-2.5		3.43	2.74		
> .50-1.5	3.80	3.46	2.44	1.74	
> .25-.50	4.57	3.31	1.74		
> .10-.25	4.92	3.10			
> .05-.10	4.22				
> .01-.05	4.39				

(46) Wollaston. 2 12%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0			2.78	2.40	
> 2.5-4.0		2.78	3.18		
> 1.5-2.5		2.78	3.18		
> .50-1.5	3.32	3.80	2.96		
> .25-.50	3.43	3.32			
> .10-.25	3.96	3.26	2.48		
> .05-.10	4.37	4.00			
> .01-.05					

(47) Crocid. 12 10%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					
> 2.5-4.0					
> 1.5-2.5		2.27	2.27	2.57	2.27
> .50-1.5	2.87	3.31	3.55	3.39	3.05
> .25-.50	3.31	3.73	3.39	3.69	
> .10-.25	4.00	4.21	3.53	3.57	
> .05-.10	4.00	4.00	3.45	3.17	
> .01-.05	3.67	3.50	3.17	2.29	

(48) Attapul. 2 11%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					
> 2.5-4.0					
> 1.5-2.5					
> .50-1.5		5.14			
> .25-.50		5.54			
> .10-.25	5.31	6.22			
> .05-.10	6.16	6.39			
> .01-.05	7.05	6.51			

(49) Glass 10 (M0S) 8%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0			2.46	2.72	
> 2.5-4.0		2.97	2.99	1.76	
> 1.5-2.5		3.43	2.37	1.17	
> .50-1.5	3.88	3.91	2.76	2.46	
> .25-.50	4.34	4.02	3.69	3.37	
> .10-.25	4.43	3.88			
> .05-.10	5.90	4.19			
> .01-.05	6.77	4.63			

(50) Glass 11 (K2P) 8%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					
> 2.5-4.0		2.89	3.09	2.41	
> 1.5-2.5		3.84	3.56	2.66	
> .50-1.5	4.12	4.53	3.60	1.81	
> .25-.50	4.28	3.02	1.18		
> .10-.25	4.11				
> .05-.10	3.91	2.89			
> .01-.05					

(51) Titanate 3 8%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0			3.08	1.78	
> 2.5-4.0		3.31	2.78		
> 1.5-2.5		3.10			
> .50-1.5	3.54	2.95			
> .25-.50	3.35				
> .10-.25					
> .05-.10					
> .01-.05					

(52) Attapul. 1 8%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					
> 2.5-4.0					
> 1.5-2.5					
> .50-1.5		5.12			
> .25-.50		5.48	4.64		
> .10-.25	5.75	6.50	4.94		
> .05-.10	6.62	6.65			
> .01-.05	6.96	6.65			

(53) Talc 1 7%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0					
> 4.0-8.0					
> 2.5-4.0		3.64	3.09	2.79	
> 1.5-2.5		4.02			
> .50-1.5	4.00	4.56	3.70	3.09	
> .25-.50	4.15	3.97	3.27		
> .10-.25	4.19	3.91	2.79		
> .05-.10	3.70	3.27			
> .01-.05					

(54) Glass 12 (O2P) 7%

Diameter (μm)	Length >.01-1	>1-4	>4-8	>8-64	>64
> 8.0				1.78	0.48
> 4.0-8.0			2.32	2.17	0.48
> 2.5-4.0		2.06	2.48	2.09	0.48
> 1.5-2.5		2.49	1.95	1.94	0.48
> .50-1.5	2.83	2.83	1.91	1.84	0.48
> .25-.50	2.44	0.48	1.68		
> .10-.25	2.08	1.65			
> .05-.10	2.41				
> .01-.05					

TEXT-FIGURE 1 (*continued*).—Fiber distribution by common log of the number of particles per microgram in each of 34 dimensional categories.

(55) Glass 13 (KFP) 6%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				2.03	
>4.0-8.0			2.67	2.03	
>2.5-4.0		2.89	2.73	0.92	
>1.5-2.5		3.20	1.92		
>.50-1.5	3.19	3.48	1.52	0.92	
>.25-.50	2.96	1.22			
>.10-.25	3.30				
>.05-.10	3.27				
>.01-.05					

(56) Glass 14 (P2P) 6%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				2.00	
>4.0-8.0			1.85	2.21	1.06
>2.5-4.0		2.30	2.08	1.70	
>1.5-2.5		2.28	0.91	0.69	
>.50-1.5	2.56	2.51	1.21	0.81	
>.25-.50	1.21				
>.10-.25	2.36	1.29			
>.05-.10	1.55	0.81			
>.01-.05					

(57) Glass 15 (Y2P) 6%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.96	
>4.0-8.0			1.08	2.24	0.60
>2.5-4.0		1.94	2.12	2.16	
>1.5-2.5		2.58	1.74	1.64	
>.50-1.5	3.42	2.09	1.60	1.60	
>.25-.50					
>.10-.25	3.12	1.60		1.30	
>.05-.10					
>.01-.05					

(58) Alumin. 7 5%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				$\bar{1}.27$	$\bar{1}.64$
>4.0-8.0			$\bar{1}.19$	0.31	$\bar{1}.28$
>2.5-4.0		$\bar{2}.52$	$\bar{2}.52$	$\bar{1}.43$	$\bar{2}.15$
>1.5-2.5		0.02	$\bar{1}.19$	0.31	
>.50-1.5	0.64	$\bar{1}.15$	$\bar{1}.70$	$\bar{1}.43$	
>.25-.50	1.17	$\bar{1}.24$	$\bar{1}.08$	0.51	
>.10-.25					
>.05-.10					
>.01-.05					

(59) Glass 16 (M8S) 5%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					1.37
>2.5-4.0		2.21	2.75	2.90	
>1.5-2.5		3.17	3.43	3.59	
>.50-1.5	3.41	2.92	3.46	2.81	
>.25-.50	3.83	2.48			
>.10-.25	3.81				
>.05-.10	3.85	2.45	2.14		
>.01-.05					

(60) Talc 3 4%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0		2.81			
>1.5-2.5		4.24			
>.50-1.5	4.13	4.85			
>.25-.50	3.76	3.58			
>.10-.25	3.81	3.81			
>.05-.10	3.28	3.41			
>.01-.05		2.81			

(61) Talc 2 4%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0			2.76	2.68	
>2.5-4.0		2.58	3.21	2.56	
>1.5-2.5		3.06	2.45	2.28	
>.50-1.5	2.28	3.26	2.45	1.98	
>.25-.50					
>.10-.25					
>.05-.10					
>.01-.05					

(62) Talc 4 5%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0			3.32		
>1.5-2.5		4.19	3.32		
>.50-1.5	4.06	4.81	3.79		
>.25-.50	3.40	4.58	3.02		
>.10-.25	4.38	4.22			
>.05-.10	3.62	3.32			
>.01-.05					

(63) Alumin. 8 3%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.72	
>4.0-8.0			2.25	1.86	
>2.5-4.0		2.42	2.34	0.74	
>1.5-2.5		2.87	1.70		
>.50-1.5	2.72	2.46			
>.25-.50	2.86	2.12			
>.10-.25	2.71	1.99			
>.05-.10	2.22				
>.01-.05					

(64) Glass 21 (S1) 6%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0			1.15	1.15	1.46
>2.5-4.0		1.15	1.85	2.36	2.00
>1.5-2.5		1.15	1.63	1.46	
>.50-1.5	1.46	2.06			
>.25-.50	1.15	1.46		1.15	
>.10-.25					
>.05-.10					
>.01-.05					

(65) Glass 22 (S2) 2%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.17	
>4.0-8.0			1.47	1.17	
>2.5-4.0		1.47	1.77	2.07	1.87
>1.5-2.5		1.47	1.17	2.25	1.77
>.50-1.5		1.87	1.47		
>.25-.50					
>.10-.25					
>.05-.10					
>.01-.05					

(66) Glass 17 (M6S) 0%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5		2.45		2.85	
>.50-1.5	4.43	4.83	4.38	3.79	
>.25-.50	5.17	4.88	1.87	2.15	
>.10-.25	5.60	4.73	3.58		
>.05-.10	5.45	4.28			
>.01-.05	5.16				

(67) Glass 18 (YW) 0%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				0.89	0.34
>4.0-8.0				0.92	0.40
>2.5-4.0				0.80	0.30
>1.5-2.5				1.00	0.11
>.50-1.5				$\bar{1}.10$	0.41
>.25-.50					
>.10-.25					
>.05-.10					
>.01-.05					

(68) Crocid. 13 0%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0			2.98		
>2.5-4.0		2.98	2.98		
>1.5-2.5		4.00	3.16		
>.50-1.5	4.30	4.46	2.68		
>.25-.50	4.68	4.00			
>.10-.25	4.90	3.63			
>.05-.10	4.68	2.68			
>.01-.05	4.31				

(69) Wollaston. 4 0%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0				1.43	0.95
>4.0-8.0				1.56	1.25
>2.5-4.0		1.43	1.86		
>1.5-2.5		1.25	0.95	1.80	
>.50-1.5	1.43	2.07	1.99	1.91	
>.25-.50		1.86	0.95		
>.10-.25	0.95	0.95			
>.05-.10					
>.01-.05	0.95				

(70) Talc 5 0%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0		3.43			
>1.5-2.5		4.33			
>.50-1.5	4.62	4.98			
>.25-.50	4.56	4.30			
>.10-.25	4.27	3.90			
>.05-.10	3.97	3.60			
>.01-.05	3.90	3.13			

(71) Talc 6 0%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0			3.25		
>2.5-4.0		3.25	3.25		
>1.5-2.5		3.95	3.55		
>.50-1.5	4.63	4.89	3.55	3.25	
>.25-.50	4.83	4.43	3.25	3.55	
>.10-.25	4.68	3.95			
>.05-.10	4.65	3.85	3.55	3.25	
>.01-.05	4.10	3.25			

(72) Talc 7 0%

Diameter (µm)	>.01-1	>1-4	>4-8	>8-64	>64
>8.0					
>4.0-8.0					
>2.5-4.0					
>1.5-2.5		4.37			
>.50-1.5	4.95	5.09	3.67		
>.25-.50	5.33	4.93	3.67	3.67	
>.10-.25	5.18	4.37			
>.05-.10	5.14	4.63	3.67		
>.01-.05	4.82				

Length

TEXT-FIGURE 1 (*continued*).—Fiber distribution by common log of the number of particles per microgram in each of 34 dimensional categories.

TABLE 1.—*Summary of 72 experiments with different fibrous materials*

Expt No.	Compound	Actual tumor incidence	Percent tumor probability ± SD	Common log fibers/μg, $\leq$0.25 μm × >8 μm	Expt No.	Compound	Actual tumor incidence	Percent tumor probability ± SD	Common log fibers/μg, $\leq$0.25 μm × >8 μm
1	Titanate 1	21/29	95±4.7	4.94	37	Halloy 1	4/25	20±9.0	0
2	Titanate 2	20/29	100	4.70	38	Halloy 2	5/28	23±9.3	0
3	Si carbide	17/26	100	5.15	39	Glass 8	3/26	19±10.3	3.01
4	Dawson 5	26/29	100	4.94	40	Crocid 11	4/29	19±8.5	0
5	Tremolite 1	22/28	100	3.14	41	Glass 19	2/28	15±9.0	0
6	Tremolite 2	21/28	100	2.84	42	Glass 9	2/28	14±9.4	1.84
7	Dawson 1	20/25	95±4.8	4.66	43	Alumin 6	2/28	13±8.8	0.82
8	Crocid 1	18/27	94±6.0	5.21	44	Dawson 6	3/30	13±6.9	0
9	Crocid 2	17/24	93±6.5	4.30	45	Dawson 2	2/27	12±7.9	0
10	Crocid 3	15/23	93±6.9	5.01	46	Wollaston 2	2/25	12±8.0	0
11	Amosite	14/25	93±7.1	3.53	47	Crocid 12	2/27	10±7.0	3.73
12	Crocid 4	15/24	86±9.0	5.13	48	Attapul 2	2/29	11±7.5	0
13	Glass 1	9/17	85±13.2	5.16	49	Glass 10	2/27	8±5.6	0
14	Crocid 5	14/29	78±10.8	3.29	50	Glass 11	1/27	8±5.5	0
15	Glass 2	12/31	77±16.6	4.29	51	Titanate 3	1/28	8±8.0	0
16	Glass 3	20/29	74±8.5	3.59	52	Attapul 1	2/29	8±5.3	0
17	Glass 4	18/29	71±9.1	4.02	53	Talc 1	1/26	7±6.9	0
18	Alumin 1	15/24	70±10.2	3.63	54	Glass 12	1/25	7±5.4	0
19	Glass 5	16/25	69±9.6	3.00	55	Glass 13	1/27	6±5.7	0
20	Dawson 7	16/30	68±9.8	4.71	56	Glass 14	1/25	6±5.5	0
21	Dawson 4	11/26	66±12.2	4.01	57	Glass 15	1/24	6±5.9	1.30
22	Dawson 3	9/24	66±13.4	5.73	58	Alumin 7	1/25	5±5.1	0
23	Glass 6	7/22	64±17.7	4.01	59	Glass 16	1/29	5±4.4	0
24	Crocid 6	9/27	63±13.9	4.60	60	Talc 3	1/29	4±4.3	0
25	Crocid 7	11/26	56±11.7	2.65	61	Talc 2	1/30	4±3.8	0
26	Crocid 8	8/25	53±12.9	0	62	Talc 4	1/29	5±4.9	0
27	Alumin 2	8/27	44±11.7	2.95	63	Alumin 8	1/28	3±3.4	0
28	Alumin 3	9/27	41±10.5	2.47	64	Glass 21	2/47	6±4.4	0
29	Crocid 9	8/27	33±9.8	4.25	65	Glass 22	1/45	2±2.3	0
30	Wollaston 1	5/20	31±12.5	0	66	Glass 17	0/28	0	0
31	Alumin 4	4/25	28±12.0	2.60	67	Glass 18	0/115	0	0
32	Crocid 10	6/29	37±13.5	3.09	68	Crocid 13	0/29	0	0
33	Alumin 5	4/22	22±9.8	3.73	69	Wollaston 4	0/24	0	0
34	Glass 20	4/25	22±10.0	0	70	Talc 5	0/30	0	0
35	Glass 7	5/28	21±8.7	2.50	71	Talc 6	0/30	0	3.30
36	Wollaston 3	3/21	19±10.5	0	72	Talc 7	0/29	0	0

They represent an excellent size distribution for comparison.

Wollastonite (wollaston 1-4).—Wollastonite is a naturally occurring crystalline fiber of monocalcium silicate (*15-18*). Four separate samples of this substitute for asbestos were received from the same Canadian mine. These were graded commercially according to size by the designation A, B, D, and F. It was apparent at low-power magnification that only grade F was completely fibrous and that these fibers were relatively large.

Tremolite (tremolite 1, 2).—The second type of amphibole asbestos studied was tremolite, a material that has a close affinity to the talcs. Both of these samples were from the same lot of asbestos and were in the optimal range of size for carcinogenesis. Comparison of these fibers indicated that they were distinctly smaller in diameter than the tremolite fibers used by Smith et al. (*29*).

Amosite.—The third amphibole asbestos studied was a single sample of South African amosite from the UICC standard reference samples. No efforts were made to alter this as received, and descriptions of this sample as published should apply (*19, 21, 22*).

Attapulgite (attapul 1-2).—Of the natural fibers, the clay attapulgite was of particular interest because of its use in many household items that generate respirable dust. Two different samples of this complex hydrated magnesium silicate were obtained from sources in Attapulgus, Decatur County, Georgia. Both samples were considerably refined, and by electron microscopy they were seen to be composed entirely of short fibers of consistently small diameter (*30*). These refined clays were considered by the U.S. Bureau of Mines to be 90% or greater in purity, with the remaining 10% being quartz.

Halloysite (halloy 1-2).—Halloysite is a natural fibrous hydrated aluminum silicate, which is respirable and of minute size. The 2 samples were obtained from Dr. Walter Parham, who recovered them from the raw water supply of Hong Kong. On examination these samples were seen to have a tendency for clumping in water. In an effort to disperse the minute fibers, the second sample was sonicated and treated with sodium

hexametaphosphate. Clumping persisted in this second sample, and little different was seen between the 2 samples.

Silicon carbide (si carbide).—One metallic crystalline whisker other than alumin was prepared by the General Technologies Corporation. Silicon carbide was a single sample, which was of exceptionally fine, uniform dimension.

Potassium octatitanate (titanate 1-3).—In addition to the synthetic crystals of dawsonite, aluminum oxide, and silicon carbide, 2 samples of fibrous crystalline potassium octatitanate (titanate 1 and 2) were tested. These were obtained from two different suppliers but they represent a single source. Because of the potential carcinogenicity of metallic nickel, the control for these 2 samples was nonfibrous, finely ground nickel titanate (titanate 3).

The 72 experiments represent all of the experiments done in a single dose range and with durable minerals and particles in the respirable range. Additional controls outside of these limits are mentioned in "Results."

Fiber measurements.—An aliquot of each of the 72 experimental mineral samples was placed on a Formvar-covered, slotted grid with an opening measuring 1×2 mm. This grid was air dried and first examined under the light microscope. If the fibers appeared satisfactorily distributed, a photomontage of the entire grid was made at a final magnification of ×3,000. The slotted grid was then placed in a Siemens electron microscope, Elmiskop 1-A, and the entire grid was scanned at low magnification. From this scan, an area that seemed to represent a typical distribution of particles in the specimen was selected for counting. At a final magnification of about ×5,000-100,000, a second photomontage was made of that section of the grid selected to include particles typical of the sample. This selected area, which generally measured about 350×150 μm, was then located on the lower magnification montage of the grid and examined to determine whether the area chosen was truly representative of the entire grid. Finally, all fibers in the area were counted and measured individually. For the diameters, a comparative scale at the final magnification was used to measure magnified diameters that measured less than 1 mm. In most cases, the selected area counted included at least 1,000 fibers, but the actual number varied with the overall size of the particles.

Subsequently, with the aid of the IBM system 370 computer, assuming the fibers to be of cylindrical shape and using the density of the material, we were able to estimate the weight of the counted samples and the number of particles of a given dimension in the 40-mg dose administered. For the purpose of calculation, particles were grouped into 34 dimensional ranges as indicated in text-figure 1, and the number of particles per microgram in each category was calculated. Duplicate counts on the montages were done on most samples and were surprisingly similar, as were counts on different areas of the same montage. However, when studies of repeat samples from the original fibers were made, considerable variation in counts occurred. Clearly, the method is subject to several errors; calibration of the electron microscope, deviation of particles from the assumed cylindrical shape, and sampling errors, especially where large particles are concerned, represent the major problems. Nevertheless, the estimates are probably valid to within one order of magnitude. Consequently, the counts are reported as the common log with the characteristic of the log representing the probable limit of accuracy (text-fig. 1).

RESULTS

Controls have been discussed in previous publications (*4, 6, 9-11*), but they were approached here in a slightly different way. In addition to untreated controls we studied rats in which open thoracotomy was performed and a noncarcinogenic material was either applied to the pleura or implanted in the lung. These 3 groups (table 2) were rats from numerous experiments that were of the same species, sex, and age and that were housed in the same quarters. The incidence of clearly apparent pleural neoplasms in untreated, aged outbred Osborne-Mendel female rats was essentially nonexistent. However, a few pleomorphic sarcomas that might be confused with pleural tumors occurred in the left thorax of both treated and, to a lesser degree, untreated controls. Although these tumors involved the thickness of the chest wall, in most cases the tumors appeared to be derived either from mammary gland fibroadenoma or from suture granuloma in the subcutaneous tissues. But there remained a few tumors for which no definite origin could be determined and which were histologically comparable with pleural sarcomas. In both the experimental groups and the control groups these questionable tumors were counted as pleural sarcomas. These essentially confusing tumors observed in the controls need to be taken into account in the assessment of the carcinogenicity of the experimental materials. The incidence of pleural sar-

TABLE 2.—*Incidence of pleural sarcomas in outbred female Osborne-Mendel control rats*

Time, wk	Untreated[a]	Noncarcinogenic pulmonary implants[a]	Noncarcinogenic pleural implants[a]	Combined controls[a]
12–52	1/113	0/49	0/47	1/209
53–65	0/15	2/26	1/72	3/113
66–78	0/26	4/50	3/64	7/140
79–91	0/68	1/70	2/85	3/223
92–104	0/26	1/72	10/294	11/392
105–120	0/98	1/162	1/36	2/296
121–130	1/66	0/3		1/69
131–143	0/27			0/27
144–156	0/27			0/27
156	1/22			1/22
Total	3/488	9/432	17/598	29/1,518
Percent	0.6	2.1	2.8	1.9

[a] No. dead with pleural sarcomas/No. dead without pleural sarcomas.

comas in all 3 control groups combined, calculated by the life table method (*13*), was 7.7±4.2%. Comparison of this incidence with the pleural sarcoma incidence in the 72 individual experiments showed that the incidence of pleural sarcomas in a particular experimental group was significantly greater than that in the combined control group only if it exceeded 30% (*see* expts 1–29 in table 1).

In regard to the controls, some negative experiments with intrapleural implants not used as controls should be mentioned. These experiments included intrapleural implants that did not conform to the type of materials under consideration because the particles were either nondurable (cotton lint, gypsum, and carrageenan), were of greater than respirable size (steel shavings, steel wool, vermiculite, polyurethane, tungsten carbide, and infusorial earth), or were exclusively nonfibrous (polyacrylic nitrile, antigorite, silicon dusts, and several glasses). None of these experiments had an incidence of pleural sarcoma that was significantly greater than the 7.7% incidence of the combined control group.

From the summarization of the 72 experiments in table 1 and text-figure 1, even cursory examination of the fiber distribution suggested that particles in the relatively thin- and long-dimensional categories were associated with higher tumor probabilities. This observation was confirmed by the statistical correlation and regression techniques that were used in previous papers (*4, 9, 10*). The logit transformation (*13*) was applied to the estimated tumor probabilities (p) according to the formula: Logit$=\ln [p/(1-p)]$, where ln denotes the natural logarithm. The 34 dimensional categories indicated in text-figure 1 were arbitrarily grouped into 11 larger categories, and the simple correlation coefficients of the logit of tumor probability with the common logarithms of numbers of particles per microgram in each of these categories was calculated (*see* table 3). The maximum correlation coefficient, 0.80, was with particles equal to or less than 0.25 μm in diameter and greater than 8 μm in length. There was no correlation with particles equal to or less than 4 μm in length or with particles greater than 1.5 μm in diameter, but relatively good correlations were noted with log numbers of fibers in categories greater than 4 μm in length and up to 1.5 μm in diameter, with correlation coefficients of 0.45–0.80.

The possibility of the existence of relationships between the particle size distributions and tumor probabilities, which are not disclosed by the simple correlation coefficients in table 3, was explored by multiple regression methods. These methods were used to find the best-fitting function of the form: logit$=a + b_1 x_1 + \ldots + b_k x_k$, where $x_1, \ldots, x_k$ represent the common logs of numbers of the particles per microgram in the size categories of table 3, and $a, b_1, \ldots, b_k$ are the regression coefficients to be estimated. The analysis indicated that the addition of further dimensional categories to the category with diameter equal to or less than 0.25 μm and with length greater than 8 μm did not significantly improve the explanation of the variation in tumor probability. The regression equation for the single variable (x) representing the common log of number of particles per microgram with diameters equal to or less than 0.25 μm and lengths greater than 8 μm was:

$$\ln[p/(1-p)] = \underset{(0.24)}{-2.62} + \underset{(0.0834)}{0.9305}x.$$

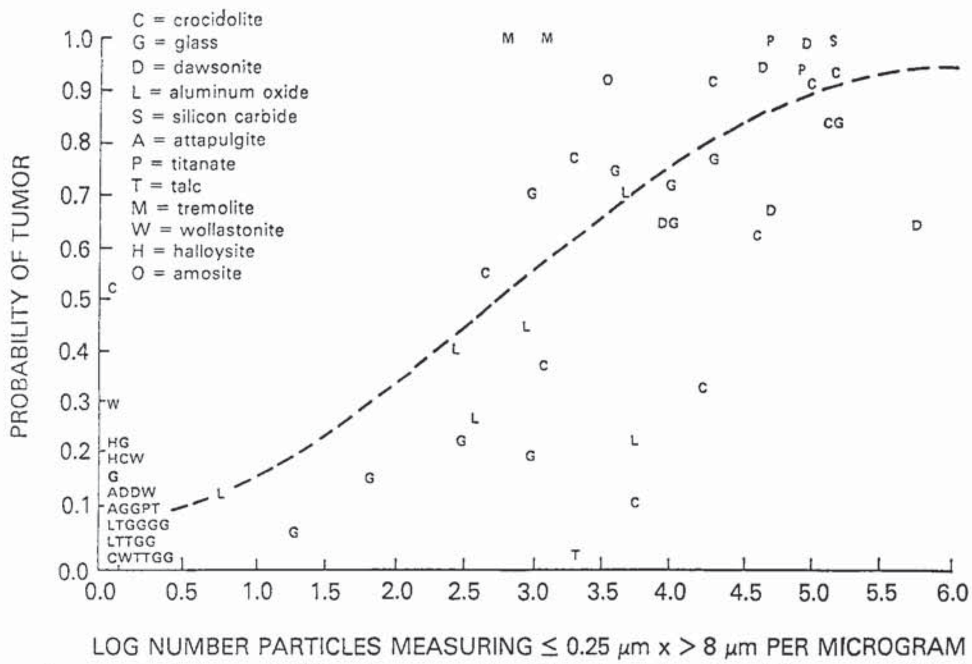
The *numbers in parentheses* beneath the regression coefficients are their estimated standard deviations. The relationship expressed by the above equation is highly significant ($P<0.0001$). The estimated regression curve is illustrated in text-figure 2.

The fact that the use of additional dimensional categories did not significantly improve the fit of the regression equation does not indicate lack of carcinogenicity in other categories. The regression of logit of tumor probability on common log of numbers of particles in other categories with a diameter up to 1.5 μm and a length greater than 4 μm would also indicate a highly significant relationship. The difficulty here is that the numbers of particles in adjacent size categories were highly correlated. Better definition of the critical range of carcinogenicity would require more narrowly defined samples (i.e., particles in a narrower dimensional range). What is perhaps more likely than the existence of a narrow range of sizes within which particles are carcinogenic and outside of which they are not is that the probability of tumor falls as particle diameter increases and length decreases.

Of the 72 experiments, 7 had tumor incidences that deviated markedly from those predicted by the estimated regression line. These were: experiments 5 (tremolite 1), 6 (tremolite 2), 26 (crocid 8), 29 (crocid 9), 33 (alumin 5), 47 (crocid 12), and 71 (talc 6) (*see* table 1 and text-fig. 2). For the first 3 of these experiments the observed responses were higher than the predicted responses, but the high responses can in part be explained by the fact that there were substantial numbers of fibers in size categories adjacent to the category used in the regression equation. For the remaining 4 experiments, the observed response was substantially lower than the expected response; although no apparent explanation existed for these deviations, they were possibly due to inaccuracies in the assessment of functional particle size. In preparations of amphibole asbestoses (which included the crocidolites and tremolites), we observed that both

TABLE 3.—*Correlation coefficients of logit of tumor probability with common logarithm of number of particles per microgram in different dimensional ranges*

Fiber diameter μm	Fiber length, μm ≤4	>4–8	>8
>4	—	−0.28	−0.30
>1.5–4	−0.45	−0.24	0.13
>0.25–1.5	0.01	0.45	0.68
≤0.25	0.20	0.63	0.80



TEXT-FIGURE 2.—Regression curve relating probability of tumor to logarithm of number of particles per μg with diameter ≤0.25 μm and length >8 μm.

clumping and fragmentation of the particles were greater than those in the other minerals, and estimates of particle size distribution in duplicate samples varied most for amphibole asbestoses.

DISCUSSION

The results show that a wide variety of compounds that seem to have only dimension and durability in common are carcinogenic for the pleura of the rat. Our conclusions regarding those dimensional categories that correlate strongly with probability of pleural tumor remain essentially the same as in previous studies, namely, that probability of pleural sarcoma correlates best with fibers that measure ≤0.25 μm×>8 μm, but that relatively high correlations were also observed with fibers in other categories having a diameter up to 1.5 μm and a length greater than 4 μm. A more refined estimate of critical carcinogenic dimension may be possible if the parameters of the experiments were changed. A different animals species, lower dose, more precise means of fiber measurement, more accurate volumetric calculations, and samples with narrower dimensional ranges all might be determining factors in better assessment of the particle dimensions critical to carcinogenicity. However, we should keep in mind two points: *a*) the dimensional limits are probably far from absolute, and *b*) we are dealing with cancer in the rat and thus extrapolation to man may not be precise.

It is clear from the histologic studies of these experiments and of previous studies that our data offer an explanation more for the lack of carcinogenicity of short fibers and thick fibers than for the carcinogenicity of long, thin fibers. Sections of preneoplastic pleural lesions show avid phagocytosis of both short fibers and large-diameter fibers but negligible phagocytosis of long, thin fibers. Consequently, in these experiments we may simply be measuring the efficiency of phagocytosis. Doubtless, we have little real knowledge of the way that long, thin fibers can cause cancer, but as Rous (*31*) once said, "Since what we think largely determines what we do, it is well that we think something." In the spirit of this quote, it might be profitable to consider potential mechanisms of cancer production by long, thin fibers. Of first importance are those hypotheses in which the progenitor of the cancer cell is not directly affected by the fiber. The long latent period would suggest that a generalized alteration either in local milieu or systemic environment might be at fault. In this regard, the abundant collagen in the preneoplastic pleural scars should be noted. Consideration of a relationship between this phenomenon and "solid-state" carcinogenesis is attractive, though the reduction of plastic sheets to small particles tends to reduce carcinogenesis. Mechanisms of solid-state carcinogenesis have been thoroughly reviewed by Brand (*32*), and little more need be added.

Any hypothesis concerning fibers must take into account the fact that both short fibers and thick fibers are less carcinogenic than fine, long fibers. Since dose was fixed in weight, but was different in dimension for all experiments, one might consider the surface area as a possible factor. If this were the case then fibers from the same pool that were modified only by shortening should be equal in tumor-producing capacity. Clearly, this is not true in the following experiments: 13 (glass 1, MOL) vs. experiment 49 [glass 10, MOS *see* (*4, 10*)],

and in experiment 24 (crocid 6) and experiment 25 (crocid 7) vs. experiment 40 (crocid 11), experiment 47 (crocid 12), and experiment 68 (crocid 13). However, in these examples the phagocytosis variable cannot be ruled out.

A provocative explanation relates to the ability of fine, long fibers to penetrate cells without killing them. That this can occur is evident from in vitro studies (*33*). However, simple penetration of cells by mycelia of fine dimension (a notable aspect of contamination of cell cultures by fungi) rarely produces transformation of cell cultures and thus is unlikely to produce cancer. However, mineral fibers differ from fungi in their rigidity as well as chemical content, and one easily could conceive of physical differences between the mineral fibers and mycelia that might be critical.

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